

THE HEMOGLOBIN MOLECULE IN HEALTH AND DISEASE

LINUS PAULING

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THE HEMOGLOBIN MOLECULE IN HEALTH AND DISEASE

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(Read April 19, 1951)

I. THE HEMOGLOBIN MOLECULE IN HEALTH

HEMOGLOBIN is one of the most interesting chemical substances in the world—to me it is the most interesting of all. Each of us carries around with him his own supply, amounting to a pound or two, approximately one per cent of the body weight. This supply is in the red corpuscles of the blood. Hemoglobin is the pigment of blood: it has a beautiful red color in arterial blood, and a purple color in venous blood. It is hemoglobin that gives a pink flush to our skin; we are pale when there is a deficiency of hemoglobin in the skin, either because of a general deficiency of the substance in the body, an anemia, or because blood is driven from the skin to the interior of the body by the contraction of the blood vessels in the skin.

The red corpuscles in man are flattened disks about 70,000 Å in diameter and 10,000 Å thick. In an ordinary microscope they have the appearance shown in figure 1. These red cells are suspended in the plasma of the blood, and they constitute about one third of the blood. They are full of hemoglobin, which makes up about 35 per cent of each red cell. There are about 100 million hemoglobin molecules in each red cell; this number is small in part because the red cell itself is small, and in part because the molecules of hemoglobin are large. Their molecular weight is 68,000, which may be compared with 18 for water, 46 for ethyl alcohol, and 342 for sucrose. The molecule contains about 10,000 atoms, of carbon, nitrogen, hydrogen, oxygen, sulfur, and other elements. There are four atoms of iron in the molecule, which play a special part in the principal function of hemoglobin, that of combination with oxygen.

The main work done by the blood is that of carrying oxygen from the lungs to the tissues, and carbon dioxide and other products of breakdown of tissues and foods to the lungs and excretory organs. The hemoglobin molecule is involved in carrying oxygen from the lungs to the tissues and in carrying carbon dioxide from the

tissues to the lungs. The hemoglobin molecule can combine with four molecules of oxygen; the resultant oxyhemoglobin is bright red in color. In the tissues, where the partial pressure of oxygen is less than in the lungs, it gives up part of its load of oxygen, which then is used in oxidation reactions of various sorts. The carbon dioxide produced by oxidation of compounds containing carbon is then carried by the blood back to the lungs, and released in the exhaled air.

The four oxygen molecules that can be taken up by the hemoglobin molecule attach themselves to the four iron atoms that are present in the molecule. These iron atoms are present as the central atoms in complexes called hemes, with the structure shown in figure 2. These four flat groups of atoms are present in the hemoglobin combined with the rest of the molecule, a protein called globin. It is the hemes that are responsible for the color of hemoglobin. The nature of the bonds in the hemoglobin molecule has been elucidated in considerable part by the study of the magnetic properties of hemoglobin. It was discovered over fifteen years ago¹ that venous blood is paramagnetic—that is, it is attracted into a magnetic field—whereas arterial blood is diamagnetic, and these magnetic properties have been found to be closely correlated with the bonding of the iron atoms.

Although some of the carbon dioxide that is carried by the blood from the tissues to the lungs is in chemical combination with hemoglobin, most of it is carried in solution in the blood. The hemoglobin contributes to this mechanism of transport of carbon dioxide in a very ingenious manner. There are in the hemoglobin molecule four acid groups which are coupled with the heme groups in such a way that their acidity is greater for an oxygenated heme than for a deoxygenated heme. Accordingly when the blood reaches the

¹ Pauling, L., and C. D. Coryell, The magnetic properties and structure of hemoglobin, oxyhemoglobin, and carbonmonoxyhemoglobin, *Proc. Nat. Acad. Sci.* 22: 210–216, 1936.

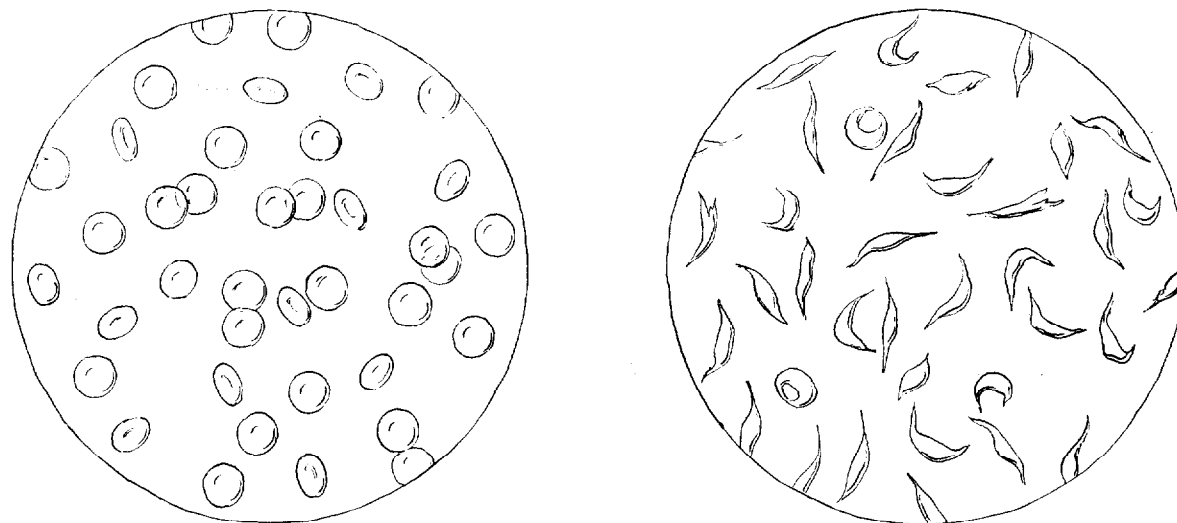


FIG. 1. At the left a drawing of normal human red cells, as seen through the ordinary microscope; at the right, sickled red cells, present in the venous blood of patients with sickle-cell anemia.

lungs, and oxygen molecules attach themselves to the iron atoms of the four heme groups in the hemoglobin molecule, the acid groups coupled with these heme groups become stronger, and liberate hydrogen ions. This increase in acidity of the blood causes some of the bicarbonate ion dissolved in the blood to change to carbonic acid, H_2CO_3 , which then breaks down to water and carbon dioxide. The heme-linked acid groups

assist in this way in the liberation of carbon dioxide in the lungs. Similarly when the blood containing oxyhemoglobin reaches the tissues, and the oxygen is liberated from the hemoglobin, the acid groups become weaker, and the blood becomes more basic, thus increasing the solubility of carbon dioxide in the blood, and assisting in removing it from the tissues.

It may well be that the hemoglobin molecule carries out other functions, but not so much is known about them as about these functions of assisting in the transport of oxygen from the lungs to the tissues and of carbon dioxide from the tissues to the lungs.

There are many different kinds of hemoglobin. All vertebrate animals and many invertebrate animals use hemoglobin as an oxygen carrier, and the hemoglobin molecule is, so far as is known, different for every animal from that of every other animal. The differences may be small, but they are detectable by the sensitive methods of examination of crystals of the substances, and testing with antisera that are produced by injection of hemoglobin of different sorts into animals. The differences are due entirely to the protein part of the molecule, the globin; the heme is the same in all hemoglobins that have yet been investigated.

In some animals, including man, a different sort of hemoglobin is present in the blood of the fetus from that in the blood of the mature animal. Human fetal hemoglobin makes up all of the blood in

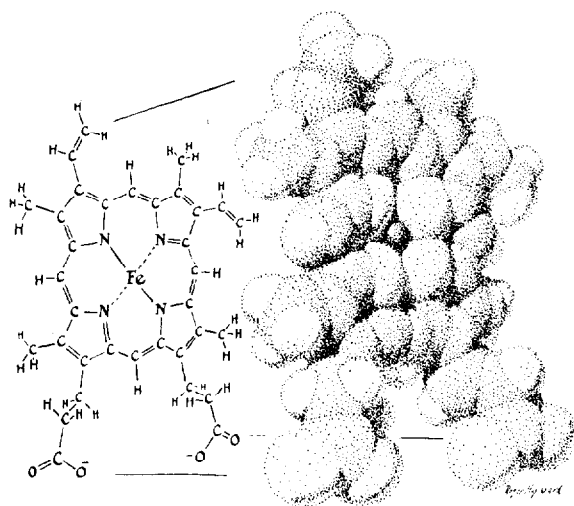


FIG. 2. A heme group, the compound of an iron atom and a protoporphyrin molecule. Four of these hemes are present in the hemoglobin molecule; they are responsible for the red color of hemoglobin, and also are involved in the combination of hemoglobin with oxygen.

the human fetus until about two months before birth. At this epoch there usually begins to appear some adult hemoglobin, which normally has completely replaced the fetal hemoglobin by two months after birth.

Our information about the nature of hemoglobin is due to many investigators. The striking sigmoid shape of the oxygen equilibrium curve was discovered by Barcroft, and the first attempts to explain it were made by A. V. Hill. The molecular weight of hemoglobin was determined by Adair, through the measurement of the osmotic pressure of a hemoglobin solution. The effects of the heme-linked acid groups were discovered by Bohr and Hasselbalch. The structure of heme was elucidated by Hans Fischer, and the nature of the bonds between the iron atom and the surrounding atoms was determined by magnetic investigations.¹ In recent years much information about the hemoglobin molecule has been obtained through the x-ray and optical investigations of Perutz and his co-workers. The identification of the groups in the globin that are adjacent to the iron atoms of the hemes, as imidazole rings of histidine side chains, was made by J. B. Conant. Measurements of the heat of oxygenation of hemoglobin solutions, made by J. Wyman, Jr., have been valuable in this identification.

II. THE HEMOGLOBIN MOLECULE IN DISEASE

Until recently it was thought that all adult human beings had the same kind of hemoglobin molecules in their red cells. Then it was discovered² that an abnormal form of hemoglobin is present in the red cells of people suffering from the disease sickle-cell anemia, and more recently still other abnormalities have come to light.

Sickle-cell anemia is a hereditary disease that is prevalent among Negroes. It is characterized by the extraordinary aspects of the red cells in the venous blood. The red cells in fresh arterial blood seem to be normal; those in venous blood, or in arterial blood kept for some time away from contact with air, or to which an agent that removes oxygen has been added, have an abnormal form, as shown in figure 1. They are twisted into crescent or sickle-like shapes, with longest dimension considerably greater than that of the normal cell. They become pleochroic, indicating

that the hemoglobin molecules have been oriented, and they are quite rigid—the normal cell is almost jelly-like in its flexibility, but when sickling occurs the cell loses this flexibility, so that it has been described as appearing to be as rigid as a crystal of ice as it moves about and abuts against fixed objects. These distorted cells, which seem also to be sticky, have difficulty in passing through capillaries, many of which are so small as just to allow passage of normal erythrocytes in single file. When sickling becomes enhanced, in a crisis of the disease, the capillaries become jammed with red cells, and the flow of blood is prevented. The interference with the flow of blood leads to anoxia, and consequent damage to the tissues. All of the clinical manifestations of the disease seem to be due to this effect. These clinical manifestations include pains in the bones and joints, kidney damage, damage to other organs, poor circulation in the extremities leading to chronic indolent skin ulcers, and poor development of the extremities. The malformed red cells tend to be removed from the circulation by the spleen and leucocytes, and this removal of the red cells leads to the characteristic anemia. The spleen becomes small and fibrotic because of numerous thromboses, so that after several crises of the disease there is little circulation of the blood through it.

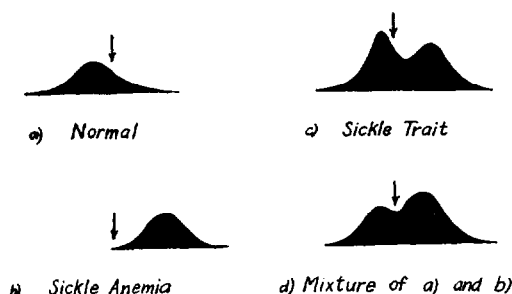
From this description of the disease it would seem that it involves a pathology of the red cell, and is to be considered, like other diseases, to be a cellular disease. However, the extraordinary fact that sickling occurs in the venous blood and not in the arterial blood suggested strongly that the hemoglobin molecule is involved. This conclusion was given greater probability by the fact that sickle-cell-anemia blood saturated with carbon monoxide does not contain sickled cells, even in the absence of oxygen; carbon monoxide combines with hemoglobin, to form carbonmonoxy-hemoglobin, which is closely similar to oxyhemoglobin in nature, whereas the other properties of carbon monoxide are much different from those of oxygen. As a result of these considerations a careful study was made of the contents of red cells from sickle-cell-anemia patients, in order to see whether or not differences in properties of the hemoglobin present in these red cell contents and normal hemoglobin could be detected. This investigation led to the discovery that the red cells of patients with sickle-cell anemia contain an abnormal hemoglobin, and no normal adult human hemoglobin.² Sickle-cell anemia was in this way

² Pauling, L., H. A. Itano, S. J. Singer, and I. C. Wells, Sickle cell anemia, a molecular disease, *Science* 110: 543-548, 1949.

found to be a molecular disease, involving a pathological hemoglobin molecule; it is the first disease to be clearly characterized as a molecular disease.

The property used to show the difference between sickle-cell-anemia hemoglobin and normal adult hemoglobin was its electrophoretic mobility—the motion of molecules, in aqueous solution, in an applied electrical field, as determined with use of the Tiselius electrophoresis apparatus. The electrophoretic patterns for normal adult human hemoglobin, sickle-cell-anemia hemoglobin, sickle-cell-trait hemoglobin, and a mixture of normal adult human hemoglobin and sickle-cell-anemia hemoglobin are shown in figure 3. Under the conditions of this study (phosphate buffer of 0.1 ionic strength and pH 6.90), the molecules of normal adult hemoglobin have a negative charge, and move toward the anode, whereas those of sickle-cell-anemia hemoglobin have a positive charge, and move toward the cathode. The difference in electrical charge amounts to about three electronic units per molecule, and corresponds to a difference in isoelectric point of 0.2 pH units.

The third pattern in figure 3 is that of the red-cell contents of a person with sickle-cell trait, a carrier of the disease sickle-cell anemia. These people are not ill—they do not show the symptoms of sickle-cell anemia, nor do they have in their venous circulation any large number of sickled cells. Their red cells can be made to sickle, how-



Carbon Monoxide Hemoglobins in Phosphate Buffer pH 6.90

FIG. 3. The electrophoretic patterns for (a) normal adult human hemoglobin, (b) sickle-cell-anemia hemoglobin, from the red cells of patients with sickle-cell anemia, (c) sickle-cell-trait hemoglobin, which is indicated to be a mixture of normal adult human hemoglobin and sickle-cell-anemia hemoglobin, and (d) a mixture of normal adult human hemoglobin and sickle-cell-anemia hemoglobin, prepared by mixing the red-cell contents from normal blood and sickle-cell-anemia blood.

ever, though not so easily as those of patients with sickle-cell anemia. It is seen from the electrophoresis pattern that their hemoglobin is a mixture of normal adult human hemoglobin and sickle-cell-anemia hemoglobin. Usually there is about 60 per cent normal adult hemoglobin, and 40 per cent sickle-cell-anemia hemoglobin, but the ratios vary rather widely.^{3, 4} Both parents of sickle-cell-anemia patients are in general found to have sickle-cell trait.

The results of the investigation of the hemoglobin of individuals with sickle-cell trait and sickle-cell anemia clarify the genetics of the disease, and lead to conclusions identical with those reached by Neel⁵ by direct genetic studies; namely, that the gene responsible for the sickling characteristic is in heterozygous condition in individuals with sickle-cell trait, and in homozygous condition in those with sickle-cell anemia. The existence of normal hemoglobin and sickle-cell-anemia hemoglobin in individuals with sickle-cell trait is, according to this postulate, a result of the presence in the cells of these individuals of an allele for normal hemoglobin and an allele for sickle-cell-anemia hemoglobin. In the cells of patients with sickle-cell anemia there are two doses of the sickle-cell allele and a complete absence of the normal hemoglobin allele, whereas in the cells of normal individuals there are two doses of the normal hemoglobin allele.

The fact that the blood of individuals with sickle-cell trait usually contains normal hemoglobin in somewhat larger amount than sickle-cell-anemia hemoglobin, the ratio of the two being somewhat different for different individuals, has been ascribed recently by Itano⁶ to a genetic difference of rate of manufacture of normal hemoglobin, as compared with the rate of manufacture of sickle-cell-anemia hemoglobin.

After the discovery of the existence of an abnormal form of adult human hemoglobin in sickle-

³ Wells, I. C., and H. A. Itano, Ratio of sickle-cell-anemia hemoglobin to normal hemoglobin in sicklemics, *Jour. Biol. Chem.* **188**: 65-74, 1951.

⁴ Neel, J. V., I. C. Wells, and H. A. Itano, Familial differences in the proportion of abnormal hemoglobin present in the sickle cell trait, *Jour. Clin. Invest.* **30**: 1120-1124, 1951.

⁵ Neel, J. V., The inheritance of sickle cell anemia, *Science* **110**: 64-66, 1949; The inheritance of the sickling phenomenon, with particular reference to sickle cell disease, *Blood* **5**: 389-412, 1951.

⁶ Itano, H. A., The inheritance of three molecular species of adult human hemoglobin; a paper submitted for publication.

cell-anemia patients and individuals with sickle-cell trait, two more types of abnormal adult human hemoglobin were discovered. The second abnormal hemoglobin, hemoglobin *c* (the letters *a* and *b* being used to represent normal adult human hemoglobin and sickle-cell-anemia hemoglobin, respectively), was discovered by Itano and Neel.⁷ It differs in its isoelectric point from normal adult human hemoglobin by twice as much as does sickle-cell anemia hemoglobin. Its presence in blood is easily shown by an electrophoretic experiment. Four patients, suffering with a disease that had been diagnosed as sickle-cell anemia, were found to contain in their red cells roughly equal amounts of sickle-cell-anemia hemoglobin and abnormal hemoglobin *c*. Investigation of the parents showed one parent to be an individual with sickle-cell trait: his red cells were found to contain roughly equal amounts of normal human hemoglobin and sickle-cell-anemia hemoglobin; whereas the other parent was found to be a carrier of the new abnormal hemoglobin *c*, with red cells containing roughly equal amounts of normal human hemoglobin and hemoglobin *c*. The rules of Mendelian genetics would lead to the prediction that about one quarter of the children should be of genetic type *bc*, and should contain in their red cells approximately equal amounts of sickle-cell-anemia hemoglobin *b* and the new abnormal hemoglobin *c*. The type of anemia resulting from this genetic constitution must be considered a new disease. It is similar to sickle-cell anemia in that the red cells sickle nearly as readily as those of a sickle-cell-anemia patient, and it is presumably this phenomenon that causes the clinical manifestations of the disease. The carriers of the new abnormality, like the carriers of sickle-cell anemia, are not anemic. Moreover, their red cells cannot be made to sickle.

Another abnormal form of hemoglobin, hemoglobin *d*, has also been recognized by Itano.⁸ The electrophoretic properties of hemoglobin *d* are very closely similar to those of sickle-cell-anemia hemoglobin. However, the solubility characteristics of hemoglobin *d* are different from those of sickle-cell-anemia hemoglobin, and, moreover, the cells of carriers of hemoglobin *d* cannot be made to sickle. The new disease, shown by in-

dividuals with sickle-cell-anemia hemoglobin and hemoglobin *d* in their erythrocytes, is similar in nature to sickle-cell anemia.

So far individuals of seven genetic types, involving hemoglobins *a*, *b*, *c*, and *d*, have been discovered: the types represented are *aa* (normal individuals), *ab* (individuals carrying sickle-cell trait), *bb* (patients with sickle-cell anemia), *ac* (carriers of the second abnormal hemoglobin *c*), *bc* (patients with the first new disease, involving the inheritance of a sickle-cell-anemia allele and an allele for the second abnormal hemoglobin, *c*), *ad* (carriers of the third abnormal hemoglobin, *d*), and *bd* (patients with the second new disease, resulting from the inheritance of a sickle-cell-anemia allele and an allele of the third abnormal hemoglobin, *d*). Individuals of types *cc*, homozygous in abnormal hemoglobin *c*, and *dd*, homozygous in abnormal hemoglobin *d*, have not yet been discovered, nor have individuals of type *cd*, carrying both of these two abnormal alleles.

Hematological abnormalities involving human fetal hemoglobin have recently been discovered. Last year it was reported by Liquori⁹ that the hemoglobin of some individuals with thalassemia major (Cooley's anemia, Mediterranean anemia) contained approximately 50 per cent normal hemoglobin and 50 per cent human fetal hemoglobin. Further studies by Alexander Rich¹⁰ led to the discovery of two patients with thalassemia major whose red cells contained 100 per cent (to within 5 per cent) of fetal hemoglobin, although these patients were past the fetal stage (age two years). Itano¹¹ has found that human fetal hemoglobin is present in small amount in the red cells of sickle-cell-anemia patients, and, together with sickle-cell-anemia hemoglobin and normal hemoglobin, in the blood of individuals who have inherited the thalassemia gene and the sickle-cell-anemia gene. There thus exists strong indication that the presence of severe anemia can cause the continued manufacture of fetal hemoglobin, in an effort to counteract the anemia. Fetal hemoglobin has also been reported by Singer, Chernoff, and Singer¹²

⁹ Liquori, A. N., Presence of foetal haemoglobin in Cooley's anemia, *Nature* **167**: 950-951, 1951.

¹⁰ Rich, A., Studies on the hemoglobin of Cooley's anemia and Cooley's trait, *Proc. Nat. Acad. Sci.* **38**: 187-196, 1952.

¹¹ Itano, H. A., The identification of fetal hemoglobin in sickle-cell anemia by electrophoretic, spectrophotometric, and solubility studies; unpublished investigation.

¹² Singer, K., A. I. Chernoff, and L. Singer, Studies on abnormal hemoglobins, *Blood* **5**: 413-435, 1951.

⁷ Itano, H. A., and J. V. Neel, A new inherited abnormality of human hemoglobin, *Proc. Nat. Acad. Sci.* **36**: 613-617, 1950.

⁸ Itano, H. A., A third abnormal hemoglobin associated with hereditary hemolytic anemia, *Proc. Nat. Acad. Sci.* **37**: 775-784, 1951.

to be present in the blood of adult patients suffering from anemias secondary to leukemia or carcinoma.

III. THE STRUCTURE OF THE HEMOGLOBIN MOLECULE

Although it has not been found possible as yet to make a complete structure determination for any hemoglobin molecule, a large amount of evidence bearing on the problem has been obtained, and many features of the structure can now be discussed with confidence.

Much of our knowledge about this molecule has been obtained through the vigorous efforts of M. Perutz and his collaborators at Cambridge University. Their x-ray studies^{13, 14} have led to the conclusion that the hemoglobin molecule (horse hemoglobin) is about 57 Å long, and that its dimensions in the other two directions are about 40 or 50 Å. Moreover, strong evidence has been obtained by Perutz that the molecule consists of rods of polypeptide chains extending in the 57-Å direction. These rods are about 10.5 Å in diameter, and they are packed together in approximate hexagonal packing. There is considerable experimental evidence supporting the suggestion¹⁵ that the rods are based upon a helical configuration of polypeptide chains, the configuration being that of the α helix, in which the coiling, with formation of hydrogen bonds between planar amide groups, is such as to correspond to about 3.7 residues per turn of the helix.^{16, 17} Strong support of this suggestion was then obtained by Perutz¹⁸ through the discovery that suitably oriented crystals of hemoglobin give an x-ray reflection with spacing 1.50 Å, representing the collaboration of successive residues in the helix, which are spaced 1.50 Å apart along the helical axis. These helical rods are indicated in figure 4, the details of their ar-

rangement being, however, hypothetical. There are only five or six polypeptide chains in the molecule, and accordingly some of the rods must be connected with one another, as a single polypeptide chain.

In addition to the polypeptide chains of the protein part of the molecule, globin, the hemoglobin molecule contains four heme groups, the structure of which is completely known. These molecules are conjugated systems, and are essentially planar in configuration. Their orientation in a crystal can be determined by measurement of the pleochroism of the crystal, since the light is absorbed only when the electric vector of the light wave has a component in the plane of the molecule. It was found by Perutz¹⁹ that in crystals of horse carbonmonoxyhemoglobin all of the heme groups lie in parallel orientations, their planes being perpendicular to the 57-Å axis of the hemoglobin molecule, which is also an axis of the crystal. We thus know that the four hemes are to be attached to the globin in such a way that their planes are normal to the direction of the polypeptide rods.

In figure 4 the hemes are shown with this orientation, but not attached at the ends of the hemoglobin molecule; instead they are shown inserted in slits between layers of globin. The most direct evidence in support of this position is provided by measurements of the combining power of hemoglobin with alkyl isocyanides.²⁰ It has been assumed for twenty years, since the suggestion by Conant, that a heme group is attached by the iron atom, on one side of the plane of the group, to an imidazole nitrogen atom of a histidine side chain, and that the oxygen molecule or other ligand attaches itself to the iron atom on the other side of the plane of the heme group. Detailed information about the nature of the bonds formed by the iron atom has been obtained through the investigation of the magnetic properties of hemoglobin and oxyhemoglobin,¹ and it is known that in hemoglobin itself the iron atom forms bonds of essentially ionic nature with adjacent atoms, whereas in oxyhemoglobin and similar compounds the iron atom forms six covalent bonds, which are directed towards the corners of an octahedron. These six bonds are formed with the four nitro-

¹³ Boyes-Watson, J., E. Davidson, and M. F. Perutz, An x-ray study of horse methaemoglobin, *Proc. Roy. Soc. A* **191**: 83-132, 1947.

¹⁴ Perutz, M. F., An x-ray study of horse methaemoglobin. II, *Proc. Roy. Soc. A* **195**: 474-499, 1949.

¹⁵ Pauling, L., and R. B. Corey, The polypeptide chain configuration in hemoglobin and other globular proteins, *Proc. Nat. Acad. Sci.* **37**: 282-285, 1951.

¹⁶ Pauling, L., R. B. Corey, and H. R. Branson, The structure of proteins: two hydrogen-bonded helical configurations of the polypeptide chain, *Proc. Nat. Acad. Sci.* **37**: 205-211, 1951.

¹⁷ Pauling, L., and R. B. Corey, Atomic coordinates and structure factors for two helical configurations of polypeptide chains, *ibid.* **37**: 235-240, 1951.

¹⁸ Perutz, M. F., New x-ray evidence on the configuration of polypeptide chains, *Nature* **167**: 1053-1054, 1951.

¹⁹ Perutz, M. F., Absorption spectra of single crystals of haemoglobin in polarized light, *Nature* **143**: 731, 1939.

²⁰ St. George, R. C. C., and L. Pauling, The combining power of hemoglobin for alkyl isocyanides, and the nature of the heme-heme interactions in hemoglobin, *Science* **114**: 629-634, 1951.

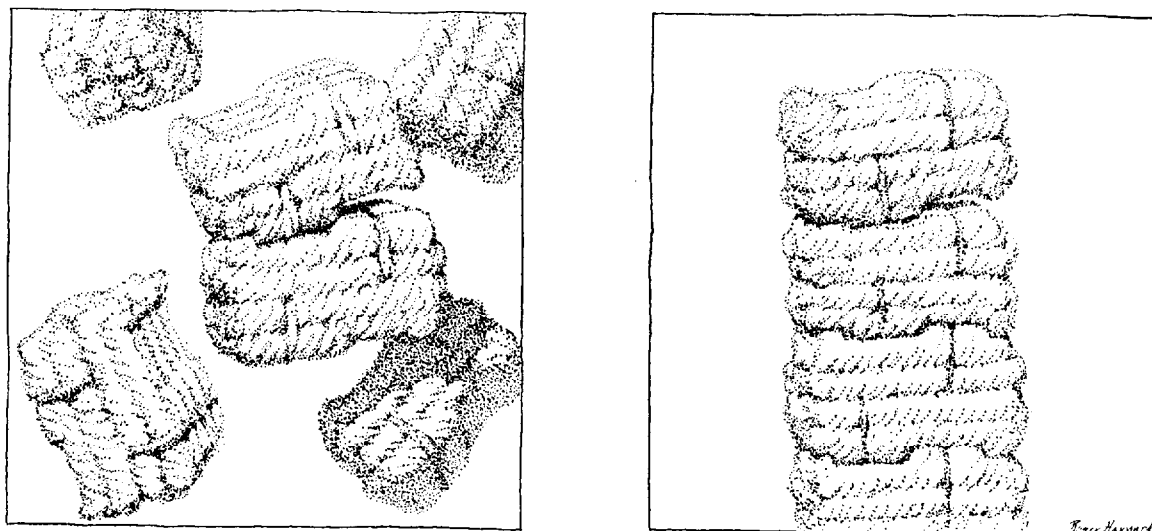


FIG. 4. A drawing indicating some of the features of the structure of the hemoglobin molecule, and the postulated mechanism of sickling of sickle-cell-anemia erythrocytes. The four hemes are indicated to be contained within slits in the hemoglobin molecule, their planes being perpendicular to the axes of the helical rods in the protein. At the right the molecules of sickle-cell-anemia hemoglobin, without oxygen attached to the hemes, are shown as having self-complementary configurations, which permit them to aggregate into long strings of molecules. At the left, the addition of oxygen or other ligand to the hemes is shown as swelling them enough to destroy the self-complementariness of the molecules, thus interfering with the formation of the aggregates.

gen atoms of the porphyrin molecule, which lie in the plane of the molecule, and with the nitrogen atom of the imidazole ring, to one side of the plane, and the oxygen molecule or other ligand, to the other side of the plane. The fact that the compounds ethyl isocyanide, isopropyl isocyanide, and tertiary butyl isocyanide show successively smaller combining powers with hemoglobin, although their combining powers with free heme groups are essentially the same, provides strong evidence that there is steric interference with the attachment of the isocyanide molecule, and this steric interference can be produced only by a part of the globin molecule. Accordingly the conclusion is reached that there is part of the globin molecule on each side of the heme group, as sketched in figure 4. Many other pieces of information are compatible with this structure, and difficult to interpret in terms of a structure in which the heme groups are attached to the surface of the globin. In particular, this model provides an explanation of the nature of the oxygen combining curve of hemoglobin—the fact that a second oxygen molecule, and a third and fourth, attach themselves to the hemoglobin molecule more readily than does the first.

We may now ask about the nature of the difference in structure of the abnormal human hemo-

globins and normal adult human hemoglobin. First, it is found that the heme groups in sickle-cell-anemia hemoglobin, which has been investigated more than hemoglobins *c* and *d*, are identical with protoheme, the heme present in normal hemoglobin. The abnormality is thus to be attributed to the globin part of the hemoglobin.

An obvious suggestion is that there is a difference in amino-acid composition of sickle-cell-anemia hemoglobin and normal adult human hemoglobin. Determination of the amino-acid composition of these two hemoglobins has, however, led to the discovery of no abnormality.²¹ A difference of one or two residues of one or another amino acid might be permitted by the analyses, but no difference is required by them. In addition, studies of the end groups have shown that there are present in sickle-cell-anemia hemoglobin, as well as in normal adult human hemoglobin, about five or six end groups with free amino groups, and that the amino acid represented, valine, is the same for sickle-cell-anemia hemoglobin as for normal adult human hemoglobin.²² The

²¹ Schroeder, W. A., L. M. Kay, and I. C. Wells, Amino acid composition of hemoglobins of normal Negroes and sickle cell anemics, *Jour. Biol. Chem.* **187**: 221-240, 1950.

²² Havinga, E., and F. C. Green, End-group analyses of sickle-cell hemoglobin; unpublished investigation.

uncertainty in this investigation is about one residue.

Electrophoretic studies on the globins obtained from sickle-cell-anemia hemoglobin and normal adult human hemoglobin by careful removal of the hemes have shown that there is a difference in electrical charge on the globin molecules approximately the same as that on the two kinds of hemoglobin.²³ If the globin is treated with guanidinium chloride in solution, and the guanidinium chloride is then removed by dialysis, the resultant globins, which might be described as denatured globins, have a changed mobility, this mobility being identical to within the experimental error for sickle-cell-anemia denatured globin and normal adult human denatured globin.²³ There is thus indication that the polypeptide chains involved in sickle-cell-anemia globin are the same as those involved in normal adult human globin, and that the difference in structure between these molecules is simply a difference in the way in which the polypeptide chains are folded. In order to account for the difference in electrical charge it is necessary to assume that the difference in folding changes the acid strength of some of the groups in the molecule. If this hypothesis is correct, we shall have to conclude that there is a gene in the cells of the human body that is responsible for the folding of the polypeptide chains, in the proper way, in the manufacture of adult hemoglobin.

In the paper announcing the discovery of sickle-cell-anemia hemoglobin² the mechanism of the sickling process was discussed in the following way.

It is likely that it is the globins rather than the hemes of the two hemoglobins that are different. Let us propose that there is a surface region on the globin of the sickle-cell-anemia hemoglobin molecule which is absent in the normal molecule and which has a configuration complementary to a different region of the surface of the hemoglobin molecule. This situation would be somewhat analogous to that which probably exists in antigen-antibody reactions.²⁴ The fact that sickling occurs only when the partial pressures of oxygen and carbon monoxide are low suggests that one of these sites is very near to the iron atom of one or more of the hemes, and that when

the iron atom is combined with either one of these gases, the complementariness of the two structures is considerably diminished. Under the appropriate conditions, then, the sickle-cell-anemia hemoglobin molecules might be capable of interacting with one another at these sites sufficiently to cause at least a partial alignment of the molecules within the cell, resulting in the erythrocyte's becoming birefringent, and the cell membrane's being distorted to accommodate the now relatively rigid structures within its confines. The addition of oxygen or carbon monoxide to the cell might reverse these effects by disrupting some of the weak bonds between the hemoglobin molecules in favor of the bonds formed between gas molecules and iron atoms of the hemes.

In the discussion of the combining power of hemoglobin with alkali isocyanides, and the postulate that the heme groups are buried within the globin of the hemoglobin molecule,²⁰ a further discussion of the nature of the process of sickling was given, as follows:

Our postulate provides an obvious explanation of the action of oxygen in preventing the sickling of sickle-cell-anemia erythrocytes. We have visualized the sickling process as one in which complementary sites on adjacent hemoglobin molecules combine. It was suggested that erythrocytes containing oxy-hemoglobin or carbonmonoxyhemoglobin do not sickle because of steric hindrance of the attached oxygen or carbon monoxide molecule. This steric-hindrance effect might be the distortion of the complementary sites through the forcing apart of layers of protein, as is suggested by the isocyanide experiments.

In figures 4 and 5 the postulated mechanism of interaction of sickle-cell-anemia hemoglobin molecules is illustrated. Because of the assumed complementariness in structure, the molecules of sickle-cell-anemia hemoglobin (without oxygen molecules or other molecules attached) could interact to form long chains of molecules. These long chains of molecules could attract one another into parallel orientation, causing the formation of a crystal or liquid crystal. Evidence has recently been obtained by Harris²⁵ in support of this picture, through the observation that solutions of sickle-cell-anemia hemoglobin, containing over 10 per cent of the protein, form liquid crystals of the nematic type, with the shape of double circular cones. Also, Perutz and Mitchison²⁶ have made

²³ Hayinga E., and H. A. Itano, Electrophoretic studies on the globins of sickle-cell-anemia hemoglobin and normal adult human hemoglobin; unpublished investigation.

²⁴ Pauling, L., A theory of the structure and process of formation of antibodies, *Jour. Am. Chem. Soc.* **62**: 2643-2657, 1940.

²⁵ Harris, J. W., Studies on the destruction of red blood cells. VIII. Molecular orientation in sickle cell hemoglobin solutions, *Proc. Soc. Exptl. Biol. Med.* **75**: 197-201, 1950.

²⁶ Perutz, M. F., and J. M. Mitchison, State of haemoglobin in sickle-cell anemia, *Nature* **166**: 677-679, 1950.

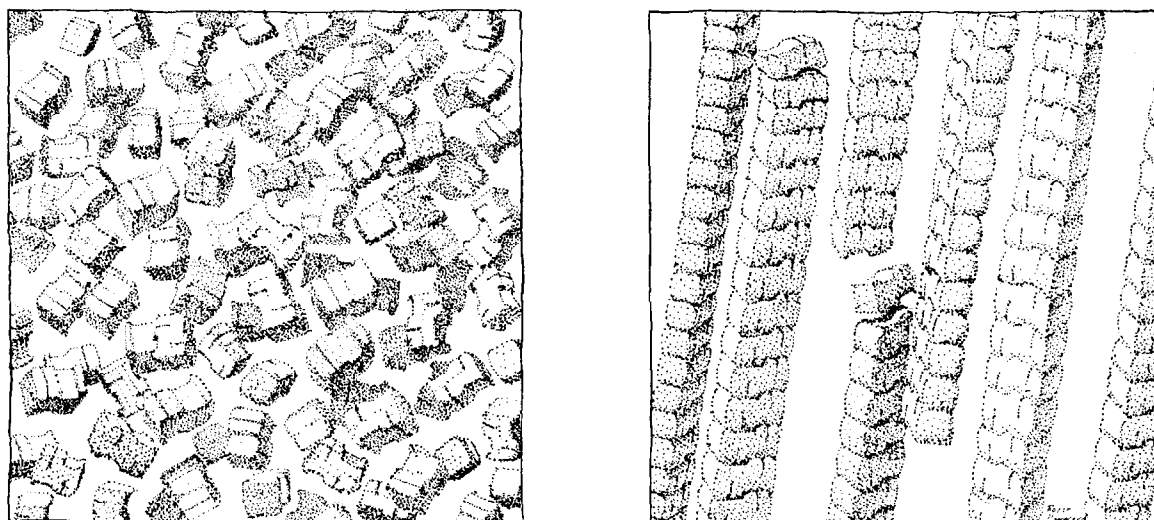


FIG. 5. At the left, molecules of normal hemoglobin or of oxygenated sickle-cell-anemia hemoglobin are shown, with random orientations, and at about the average distance apart characteristic of red-cell contents. At the right long strings of molecules of deoxygenated sickle-cell-anemia hemoglobin are shown, assuming the parallel orientation characteristic of the nematic liquid crystals that presumably form within the red cells in the venous blood of patients with sickle-cell anemia, and twist the red cells into the abnormal shape characteristic of the disease.

a quantitative study of the pleochroism of sickled cells, and have shown that the pleochroism is compatible with this postulate, the orientation of the heme groups being as indicated in figure 4, namely, the planes of the heme groups being parallel to the long axis of the sickled cell.

In the postulated mechanism, the introduction of an oxygen molecule or carbon monoxide molecule causes an effective increase in thickness of the heme groups, and, as shown in figure 4, destroys the complementariness in configuration of the surfaces of the molecule, and thus prevents the formation of linear aggregates, and the sickling of the cells.

On the basis of the available information we may surmise that the folding of the polypeptide chains in the globin of normal adult human hemoglobin is such that this complementariness in structure is not present, or at any rate is not so pronounced. The abnormal hemoglobins *c* and *d* seem to be intermediate in nature; we assume that there is an approximate complementariness shown by these molecules, which permits them to fit into the aggregates, together with sickle-cell-anemia hemoglobin molecules, although their own tendency to form aggregates is not sufficiently great to cause cells containing either of these abnormal hemoglobins together with normal adult human hemoglobin to sickle.

Thus at the present time we have a considerable amount of knowledge of the structure of the normal adult human hemoglobin molecule, and of the abnormal forms of this molecule that are responsible for three known molecular diseases. Although the knowledge of the structure of these molecules is as yet far from complete, it has led to the suggestion of possible methods of chemotherapeutic treatment of the diseases, which are now under investigation. In the course of time, through continued attack on the problem, the complete structure of the hemoglobin molecule will be discovered, and the precise nature of the abnormalities that are present in the molecules of sickle-cell-anemia hemoglobin, abnormal hemoglobin *c*, and abnormal hemoglobin *d*; we may feel confident that this knowledge will permit the deduction of improved therapeutic methods, and that in the future a similar attack on other diseases, through the determination of the structure of the molecules that are involved, can also be made.

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IV. SUMMARY

It has been discovered that, in addition to fetal human hemoglobin and normal adult human hemo-

globin, three abnormal forms of hemoglobin occur, in the red cells of certain individuals. One of these abnormal hemoglobins is associated with the disease sickle-cell anemia, and the two others are associated with two newly recognized hereditary anemias, resembling sickle-cell anemia. These diseases are to be considered as not diseases of the red cell itself, but rather diseases involving molecular abnormalities.

Although it has not yet been found possible to determine completely the structure of the molecules of any form of hemoglobin, a considerable amount of information about the structure of these molecules has been obtained. On the basis of this information it is possible to suggest a plausible mechanism whereby the abnormal hemoglobin molecules produce the clinical manifestations of the diseases.